THE EFFECT OF VARIOUS ADDITIVES ON COLOR DEGRADATION AND BROWNING OF INDIVIDUALLY QUICK FROZEN RED TART CHERRIES

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ABSTRACT

THE EFFECT OF VARIOUS ADDITIVES ON COLOR DEGRADATION AND BROWNING OF INDIVIDUALLY QUICK FROZEN RED TART CHERRIES.

by Shimon Mayak

Red tart cherries (Prunus cerasus L. var. Montmorency) frozen as individually quick frozen (IQF) fruit underwent red color loss and oxidation or browning during 8 months of frozen storage. Pretreating the cherries by dipping into 500 or 1000 ppm solution of SO_2 for one minute before freezing decreased the amount of red color loss and browning with less color loss occuring at the higher concentration. Color loss and browning in cherries pretreated with 0.1% citric acid was similar to that obtained for the control while those treated with 0.1% ascorbic acid - 0.1% citric acid showed greater color loss and browning. Cherries frozen in 60% sugar sirup showed little or no color loss or browning during storage.

Under accelerated oxidative defrosting conditions, red color loss and browning occured in all treatments. The rate and amount of color loss and browning was lowest in the cherries treated with 1000 ppm SO_2 and greatest in the ascorbic-citric treated fruit. Color loss and browning occurred at an accelerated rate in the sirup packed fruit and after 60 minutes was greater than that of the control samples.

Individually quick frozen cherries packed in 30 lbs. friction top cans retained more red color and showed less browning than those packed in 1 lb. polyethylene bags.

No SO_2 could be detected in SO_2 treated fruit either by the Monier-Williams distillation method or by treatment with cold NaOH prior to distillation.

Thirty-two to forty-four ppm of SO_2 were retained by either the control or SO_2 treated fruit held in SO_2 solutions containing 80, 160 and 300 ppm for 16 hours at 32°F before distillation.

The addition of 25 ppm SO_2 prior to defrosting under highly oxidative conditions prevented red color degradation in the control samples but did not completely prevent oxidation of phenolic compounds or browning. Twelve ppm SO_2 added prior to defrosting prevented color destruction and browning in the cherries pretreated with 1000 ppm SO_2 while 25 ppm added just before thawing resulted in the bleaching of fruit.

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By

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INTRODUCTION

The red color of red tart cherries (Prunus cerasus, L. var. Montmorency) constitutes an important quality factor readily evaluated by the consumer, and therefore has a significant effect upon the value of the product.

The loss of red color and subsequent oxidation or browning of the fruit tissue is related to the extent of bruising during harvest and the temperature at which the fruit is held. Lowering the temperature decreases the rate of color loss, but even in frozen storage color loss will continue throughout the storage period. The loss of red color can be minimized by decreasing the exposure of the fruit to air.

In the past few years there has been an interest in freezing red tart cherries as individually quick frozen fruit similar to the well established process for vegetables such as peas, lima beans, snap beans and cut corn. It is felt that this system of freezing would give a product that would have the advantages of being free flowing, reduce shipping and storage costs and permit multiple uses of the fruit by the consumer.

There is no date available on the effect of freezing and frozen storage on the quality of individually quick frozen cherries. This study was undertaken to determine the effect of pretreating the pitted fruit with sulfur dioxide, ascorbic acid and citric acid on retention of red color and quality of individually quick frozen cherries.

LITERATURE REVIEW

Roger (1940) reported that individually frozen cherries do not retain their original bright red color as well as cherries packed and frozen in 30-lbs. cans. Lee (1949) found that, when the fruit was packed in small containers and covered with 50-60% sugar sirup, the rate of freezing had little or no effect on the quality of the fruit after six months storage at 0°F.

Guadagni (1963) reported that rapid freezing of 30-lbs. cans of cherries (5+1), reduced the browning of the surface layers and the transfer of the red color from the skins to the expressed surrounding juice.

ANTHOCYANINS

The anthocyanin pigments of the red tart cherries have been identified by Li and Wagenknecht (1956) as cyanidin-3-rhamnoglucoside (antirrihinin); and cyanidin-3-diglucoside (mecocyanin). These pigments are phenolic compounds and are readily oxidized or reduced with the loss of red color. Sondheimer and Kertesz (1952) reported the oxidation of anthocyanin by hydrogen peroxide to a colorless product in strawberry juice when H_2O_2 was added. Although the formation of hydrogen peroxide has not been demonstrated, they suggested that it was formed during the oxidation of ascorbic acid catalyzed by Cu or Fe.

Meschter (1953) reported that Fe and Cu increase ascorbic acid oxidation and that the oxidation products accelerate anthocyanin destruction.

A rather different mechanism was reported by Huang (1955), in which crude enzyme extracts from Aspergilli hydrolyzed blackberry anthocyanins to anthocyanidins and sugar followed by spontaneous transformation of the aglycone to a colorless derivatives.

Wagenknecht et al. (1960) isolated crude anthocyanase from red tart cherries. It is believed to participate in the early stages of scald (red color loss) in red sour cherries through destruction of anthocyanin pigments. The enzyme required the presence of oxygen for its activity and was activated by the presence of catechol.

Scheiner (1960) reported that the anthocyanin decolorizing activity of cherry crude homogenates is almost doubled by the addition of catechol and that purified enzyme preparations are almost completely inactive unless catechol or some other o-dihydroxyphenol compound was present in the reaction mixture. Catechol oxidase activity and anthocyanin decolorizing activity were found in all the fractions tested. He suggested that the anthocyanins were non-enzymatically oxidized by the quinones produced in the enzymatic oxidation of dihydroxyphenol compounds.

Peng and Markakis (1963) using mushroom tyrosinase found that in the absence of catechol the reaction was very slow, but in the presence of catechol the rate of mecocyanin decoloriation increased rapidly.

Grommeck and Markakis (1964) showed that horseredish peroxidase oxidized cherry anthocyanins to a colorless form, and the maximum rate occured in the presence of H_2O_2 at concentration of 10^{-4} to 10^{-3} M.

Although phenolases and peroxidases were not purified from cherries, their activity has been reported in cherries, (Bedford, 1963).

PHENOLIC COMPOUNDS

The mechanism of the oxidation of phenolic and polyphenolic compounds and subsequent polymerization with the formation of brown pigments has been summerized by Mason (1959). Briefly, when catechol is oxidized in the presence of polyphenoloxidase, the first step in this oxidation produces o-benzoquinone, which may polymerize to form simple melanins.

Guadagni (1949), using the method described by Rosenblatt and Peluso (1941) for total phenolic compounds on a water extract of frozen peaches, pointed out that it is reasonable to assume that the loss in phenolic compounds as measured herein is a fairly good estimate of the degree of enzymatic browning. He found that 70-80% of the total phenol compounds present in a water extract were converted to dark brown substances.

Hillis and Swain (1959) extracted phenolic compounds from Victoria plum tree with absolute methanol and 50% methanol, and found that the absolute methanol fraction contains simple phenols like chlorogenic acid, and also mono or oligomeric leuco-anthocyanins. The aqueous mathanol contained higher polymeric forms. However, the higher polymeric forms were not necessarily insoluble in absolute methanol, but bound via hydrogen bonds to cell walls or protein in the plant material, and were only released when partial rehydration with an aqueous solvent mixture broke these bonds (Goldstein and Swain, 1963). It might suggest that extracting with water, although less selective, would provide a simple extraction procedure for studying the change in phenolic compounds due to enzymatic browning reaction.

SULFUR DIOXIDE

Sulfur dioxide and the salts of sulfurous acid have been found to be very effective in the retardation of enzymatic and non-enzymatic browning as well as preventing undesirable color and flavor changes in fruits (Joslyn, 1954) and(Ponting, 1960). SO_2 is also used to bring about the irreversible bleaching of cherries (Marashino) (Bullis, 1931) or the reversible bleaching of anthocyanin pigments (Jurd, 1964).

The chemistry of SO_2, sulfurous acid and its salts have been reviewed by Joslyn and Braverman, (1954) . Although it has been used for a long time, the mechanism involved in retardation of discoloration was not well established. Hamburger and Joslyn (1941) reported that SO_2 prevented darkening by being readily oxidized. Joslyn and Hohl (1948) suggested that it act as an antioxidant, utilizing the oxygen available. Johnson and Johnson (1952) reported that it decreased the enzyme activity and this was supported by Ponting (1960) who reported that polyphenolase activity was completely inhibited in the presence of 10 ppm SO_2 in buffered solution containing catechol. However, if the enzymatic oxidation was allowed to proceed until some quinone is formed, then the addition of 5 ppm SO_2 will not alter the enzyme activity because the SO_2 reacts with the quinone. Demair et al. (1960) reported that enzyme inactivation is reversible and is not related to SO_2 oxidation. Sastry et al. (1961) found complete peroxidase inhibition in the presence of 25 mg% SO_2 at pH 4.0.

Goodman and Markakis (1963) reported that if the enzyme and SO_2 were preincubated, lower levels of SO_2 had to be added to the juice in order to obtain inhibition similar to that of no preincubation.

Embs and Markakis (1965) reported that the immediate SO_2 inhibition of browning is due to the formation of a quinone SO_2 addition compounds and preventing polymerization of malanin pigment. No oxidation of SO_2 was found.

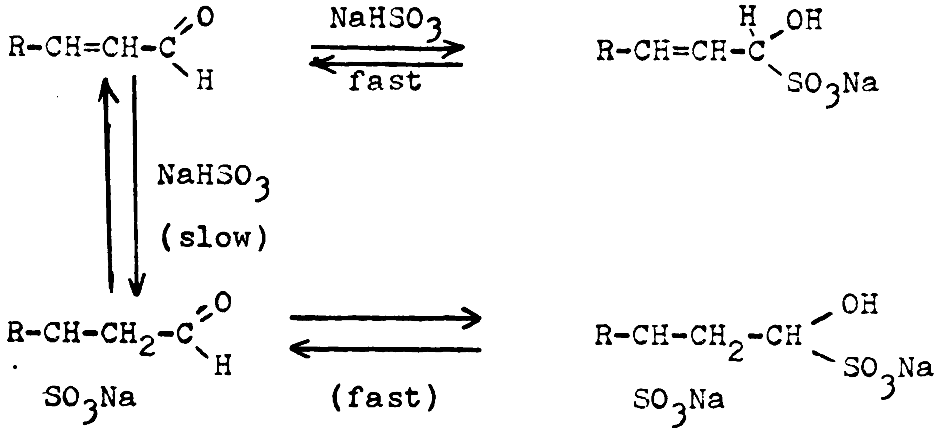
SO_2 DETERMINATION

Sulfites in fruit and vegetable tissue will exist as solution of sulfurous acid in water, as the free bisulfite ion, as the free sulfite ion, and as more or less tightly bound SO_2 in the form of the hydroxysulfonate (Joslyn, 1954). Ponting (1960) pointed out the possibility that only free SO_2

inhibits the enzyme, although almost always the total SO_2 is measured in fruit (the Monier-Williams distillation method).

It has been consistantly observed that the quantity of SO_2 in the fruit will decrease during storage (Burton et al., 1963).

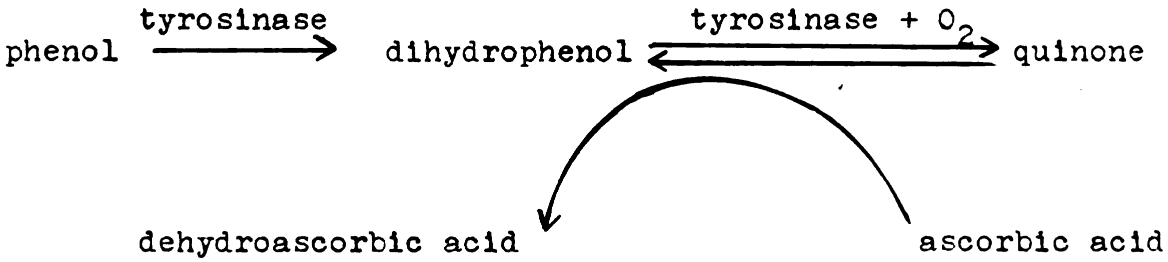
Ponting (1945) reported that the fruit texture and low temperature, employed in freezing fruit prevents penetration of SO_2 much below the surface, thus large portions of the enzyme remain potentially active, and upon thawing, the enzyme will oxidize the SO_2. There is a possibility of sulfur dioxide loss other than by oxidation to sulfate. Stadman (1948) reported loss of SO_2 from dried apricots in absence of oxygen. Burton et al. (1963) concluded, that β -sulphonaldehyde formed in the reaction of SO_2 and unsaturated aldehydes,



and since β -sulphonaldehyde will not release the β-sulphonic group under acid conditions employed in Monier-Williams distillation method it appeared to be a likely mechanism leading to a reduction in the amount of measurable sulfite in stored food stuff.

ASCORBIC ACID

Ascorbic acid has been used in many foods to preserve their color and to increase their nutritive value, (Dubois, 1949). It is generally agreed that its action as an antioxidant follows the following scheme:



It maintains the phenolic substrate in reduced form and thus prevents browning. (Robinson and Nelson, 1944), (Joslyn and Hohl, 1948), (Krueger, 1950) . Baruch and Swain (1953) and Ingraham (1956) reported that ascorbic acid might exert a partial denaturation of the polyphenoloxidase. Ponting (1960) suggested that during the oxidation reaction the enzyme is being inactivated, so that if an excess of ascorbic acid is present the enzyme eventually will be inactivated.

Weissberger (1944) reported that dehydroascorbic acid and hydrogen peroxide are formed in the first stage of ascorbic acid autoxidation with oxygen.

Hodge (1953) pointed out that quinones will oxidize ascorbic acid to dehydro-ascorbic acid, which will then undergo browning of itself. In addition, reaction between amino acids and dehydroascorbic acid will produce strongly brown colored complexes.

Tauber et al. (1935) reported the presence of a specific ascorbic acid oxidase which will catalyze only ascorbic acid oxidation.

Luther and Cragwall (1946) reported that slight adjustment in acidity by citric acid will slow the oxidation rate of ascorbic acid. However, Starachan and Moyls (1949) found that the levels of citric acid required to prevent oxidation of ascorbic acid resulted in an undesireable product.

METHODS AND MATERIALS

The cherries used in these studies were Prunus cerasus L. var. Montmorency, obtained from the Horticulture Farm at Michigan State University. The cherries were soaked for 4 hours in running water at 50°F, sorted, pitted, treated, placed in a single layer on perforated trays and frozen immediately in the -10°F freezing room, directly in front of the blower. The treatments were as follows:

1) Control: freshly pitted cherries were individually quick frozen.
2) 500 ppm SO_2: pitted cherries were dipped in 0.10% solution of $NaHSO_3$ for 1 minute, drained, and individually quick frozen.
3) 1000 ppm SO_2: pitted cherries were dipped in 0.20% solution of $NaHSO_3$ for one minute, drained and individually quick frozen.
4) Ascorbic acid - Citric acid: pitted cherries were dipped in a 1:1, ascorbic:citric solution (the solution was 0.1% was respect to both ascorbic acid and citric acid) for 1 minute, drained and individually quick frozen.
5) Citric acid - pitted cherries were dipped in a 0.1% citric acid solution for 1 minute, drained and individually quick frozen. After the cherries were completely frozen, they were transferred to 30 lbs. friction top cans. Representative samples were also packed in 1-lb. polyethylene bags.

All samples were stored at 0°F to -5°F. A representative lot of the freshly pitted cherries were also packed with 60% sirup in 303 cans, sealed and frozen at -10°F and stored at 0°F to -5°F.

Color determination

One hundred fifty gram representative samples of frozen cherries were cut into halves and blended with 150 ml distilled water in a Waring blender at high speed for 2 minutes. Representative 25 gm aliquots were removed and diluted to 150 ml. with 70% ethanol. The sample was well mixed and allowed to stand for hour and than filtered through Whatman filter paper #5. Five ml. of the extract was transferred to an Evelyn colorimeter tube followed by 5 ml. of sodium - citrate buffer (0.85 M) pH 3.4. The contents were mixed and allowed to stand for 30 minutes. Transmittancy was determined in the Evelyn colorimeter at 515 mu and the results were calculated as absorbancy units per gram of frozen cherries. Preliminary studies indicated that the alcoholic solution extracted the anthocyanin and phenolic compounds and at the same time prevented further degradation (table 1). The color also remained stable for 75 minutes after the buffers were added (table 2).

Table 1: Stability of red color values in alcoholic solution

Time after alcohol added (min)	Blending time (min) 0		30	
	A_{515mu}/gm frozen cherries			
		Difference		Difference
	(pH 3.4)	(pH 3.4 - pH 5.0)	(pH 3.4)	(pH 3.4 - pH 5.0)
120	1.255[1]	0.650	1.022	[illegible]
150	1.267	0.669	0.993	[illegible]
180	1.277	0.664	0.996	[illegible]
200	1.272	0.665	0.986	[illegible]

(1) values are averages of 4 replicates.

Table 2: Stability of red color values at pH 3.4 and pH 5.0.

Time after buffer added (min) \ Blending time (min.)	0		15		30		60	
	$A_{515\ mu}$/g frozen cherries							
	(pH 3.4)	(pH 5.0)	(pH 3.4)	(pH 5.0)	(pH 3.4)	(pH 5.0)	(pH 3.4)	(pH 5.0)
15	1.185[1]	0.609	1.099	0.581	0.967	0.559	0.660	0.456
30	1.180	0.612	1.092	0.581	0.960	0.552	0.660	0.458
45	1.190	0.612	1.104	0.586	0.972	0.547	0.674	0.458
60	1.176	0.612	1.082	0.576	0.955	0.537	0.653	0.454
75	1.176	0.609	1.078	0.576	0.944	0.537	0.643	0.454
90	1.162	0.612	1.073	0.576	0.936	0.537	0.636	0.447

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[1] Values are averages of 2 replicates

Comparisons were made on representative samples of all treatments at the end of 8 months storage period between cherries packed in 30 lbs. friction top cans and 1 lb. polyethylene bags. Absorbancy values were determined at one pH (3.4) and as a difference between two pH's (pH 3.4 - pH 5.0), according to the procedure recommended by Sondheimer and Kertesz (1948).

The rates of anthocyanin degradation and oxidation of phenolic compounds under accelerated oxidative defrosting conditions were determined using the procedure described by Guadagni et al. (1949), with the exception that the temperature was not raised to 70°F. Representative samples were also blended with 12 and 25 ppm SO_2 to determined the effect of added SO_2 on the rate of oxidation.

Total Phenolic Compounds

The total phenolic compounds were determined on the alcoholic extract using the method described by Rosenblatt and Peluso (1941). Transmittancy was measured at 660 mu using the Evelyn colorimeter. The absorbancy curve of the blue color produced, using the Bausch and Lomb 505 spectrophotometer showed no peak in the range of 640 mu to 825 mu.

Sulfur Dioxide Determination

SO_2 content was determined by the Monier-Williams distillation method (A.O.A.C. 1960). 50 gm of frozen cherries were transferred to the distillation flask, followed by 300 ml. distilled water and 20 ml. concentrated HCl and distilled into

3% H_2O_2 long enough to allow 30 minutes boiling time. The possible presence of "bound" sulfur dioxide was determined by incubating 50 gm of cherries with 100 ml (0.25 N) NaOH for 30 minutes, acidifying and distilling for 60 minutes. (Distillation times up to 120 minutes did not increase the yield of SO_2 in either procedures.)

Representative 50 gm samples of the control and cherries pretreated with 1000 ppm were held in 100 ml of SO_2 solution of different concentrations for 16 hours at 32°F. Then the SO_2 content was determined by distillation to obtained data on the amount of SO_2 that could be recovered. Blank determination were made on each of the SO_2 solutions.

RESULTS AND DISCUSSION

Color changes during frozen storage

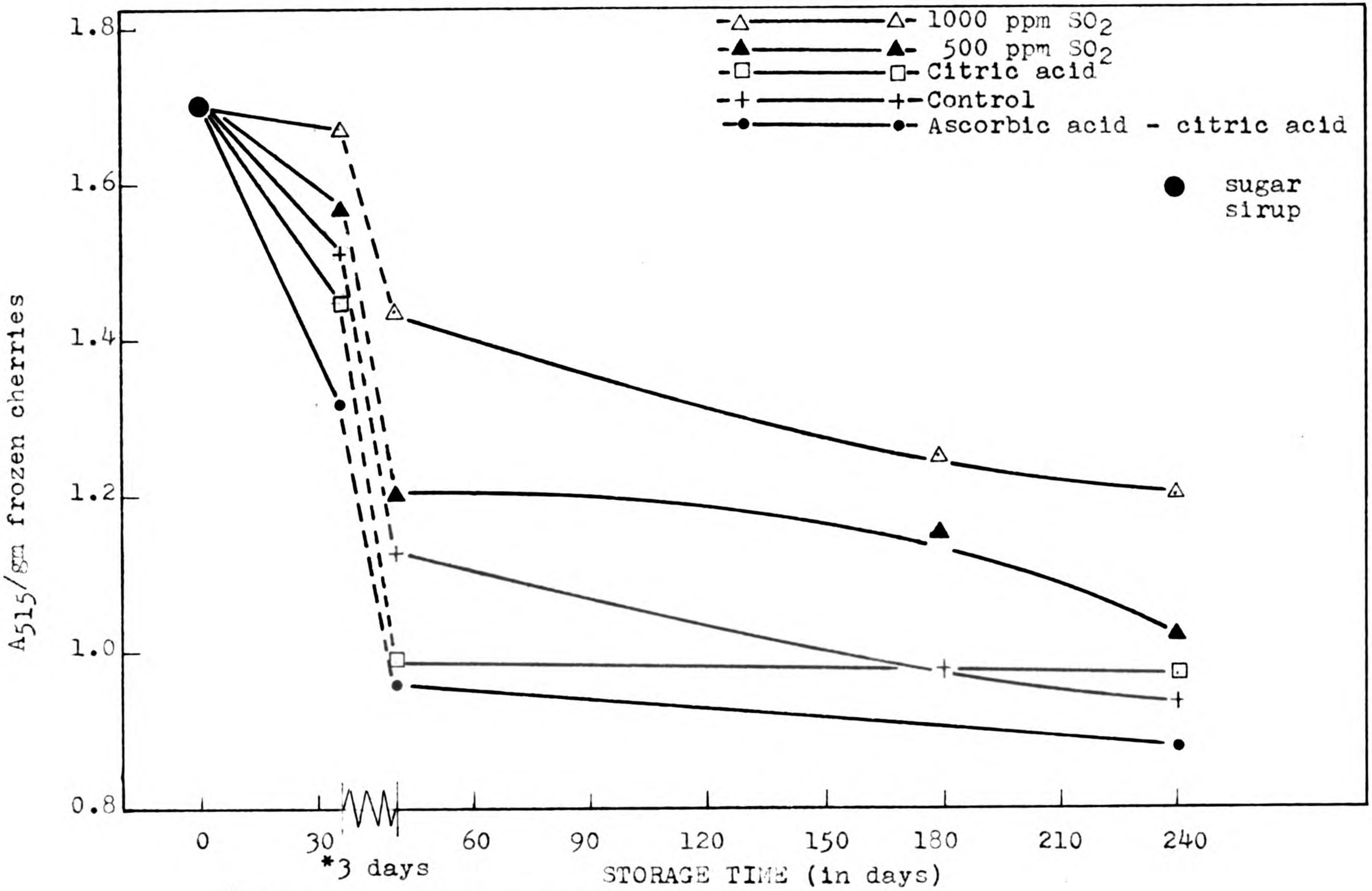
The most readily observable visible changes in pitted red cherries frozen without any pretreatment or packing medium were the loss of red color and browning of the flesh.

Color loss occurs throughout the storage period in the treatments (figure 1) and (data appendix table 3) The cherries pretreated with 1000 ppm SO_2 showed the least red color loss, followed by those treated with 500 ppm SO_2. The citric acid treatment was similar to the control, ascorbic acid - citric acid treated samples had greater color loss than those of the control. Sondheimer and Kertesz (1952) suggested the formation of H_2O_2 during ascorbic acid oxidation would rapidly oxidize anthocyanin pigments to a colorless product. Since the cherries were not protected from exposure to oxygen, rapid oxidation of ascrobic acid might be expected.

Unfortunately during the storage period a breakdown of the freezer occured, the freezer temperature reached 40°F before repairs were made and the temperature again reduced to -5°F. Although such storage conditions are unusual, it has been pointed out by Munter (1953) that the temperature of frozen fruit during transportation, storage and distribution might reach as high as 40°F.

During the period the cherries were defrosted and refro-

Figure 1. Absorption curves of red color values during storage, pH 3.4.



* Defrosting due to breakdown of the freezer

zen, the red color loss in the SO_2 treated fruit was much less than in the control or other treatments. Peng and Markakis (1963) reported that the breakdown of the anthocyanin pigments was mainly by reaction with quinones, the oxidation products of phenolic compounds. The increase in enzymatic activity during the rise of temperature would result in increased formation of quinones, which would be available to react with the anthocyanins. Embs and Markakis (1965) indicated that SO_2 reacts with quinones to form addition compounds. This would reduce the amount of quinones free to react with the anthocyanin pigments in the SO_2 treated fruit, and therefore reduce the amount of red color destruction. The relative rates of red color loss during accelerated oxidative defrosting conditions after various periods of storage are given in table 3. The relative rates depend upon and vary with the red color levels (substrate concentration).

The control and the ascorbic - citric acid pretreated cherries showed the highest color loss rate during accelerated defrosting conditions after 30 days of frozen storage with about 38 and 46% loss of red color after 30 minutes of blending. (table 3). The lowest red color loss rate occured in the fruit pretreated with 1000 ppm SO_2. The relative red color loss rates decreased in the control, ascorbic - citric and citric acid treated fruit during storage. The rates increased slightly in the SO_2 treated cherries with blending time which is in agreement with the results reported by Goodman and

Markakis (1965), who observed increasing rates of phenolic compounds oxidation with time at low concentrations of SO_2. Quinone formation during storage in excess to those bound with SO_2 will result in increasing discoloration rates.

Table 3: Color loss under accelerated oxidative defrosting conditions for the various treatments and storage time.

Time (days)	Blending time (min.)	Control		SO_2 500 ppm		SO_2 1000 ppm		Ascorbic-citric acid		Citric acid	
		A_{515}*	% loss**	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss
30	0	1.510		1.562	8.12	1.664	2.12	1.[illegible]	50	1.453	14.53
	15	1.184	30.35	1.517	10.76	1.645	3.23	0.973	42.76	1.401	17.59
	30	1.056	37.88	1.446	14.94	1.625	4.41	0.921	45.82	1.248	26.59
*** % loss during blending		30.07		7.43		2.34		29.32		14.11	
34	0	1.126	33.76	1.203	29.23	1.433	15.70	0.973	42.76	0.989	41.89
	15	1.043	38.65	1.101	35.23	1.357	20.12	0.909	46.53	0.974	42.70
	30	1.018	40.12	1.043	38.65	1.338	21.29	0.768	54.82	0.926	45.53
% loss during blending		9.59		13.30		6.63		21.07		6.37	
180	0	0.974	42.70	1.171	31.12	1.296	23.76				
	15	0.955	43.82	1.090	35.88	1.238	27.18				
	30	0.915	46.35	0.960	43.53	1.104	35.06				
% loss during blending		6.06		18.02		14.82					
240	0	0.931	45.23	1.013	40.41	1.200	29.41	0.883	48.06	0.973	42.76
	15	0.840	50.59	0.979	42.41	1.171	31.12	0.806	52.56	0.934	45.06
	30	0.811	52.29	0.970	42.94	1.133	33.35	0.701	58.76	0.864	49.18
% loss during blending		12.39		4.25		5.58		20.61		11.20	

* Absorbancy at 515 mu calculated per gram frozen cherries.

** % loss calculated as related to initial color value of pitted cherries after freezing (1.700).

*** % loss during blending calculated as related to initial color at 0-time blending.

The oxidation of the phenolic compounds was not determined for all storage periods, but the results obtained for two of them 34 and 240 days, (table 4) showed the same trend as red color destruction.

Table 4: Effect of various treatments on the oxidation of phenolic compounds during frozen storage, and subsequent accelerated oxidative defrosting conditions.

Storage time (days)	34			240		
Blending time (min.)	0	15	30	0	15	30
Treatments	A_{660mu}/gm frozen cherries					
Control	4.416	4.256	3.880	3.660	3.348	3.312
SO_2 500 ppm	4.672	4.576	4.224	4.032	3.720	3.672
SO_2 1000 ppm	5.326	4.960	4.956	4.260	4.080	3.960
Ascorbic-citric	4.366	3.952	3.520	3.648	3.432	3.024
Citric	3.904	3.632	3.312	3.216	3.036	3.060

The values are averages of 4 replicates

Sondheimer and Kertesz (1948) stated that measuring absorbancy at one pH as a measure of red pigment content is not very accurate, particularly at an advance degree of oxidation, because of the increasing proportion of brown pigments which contribute to the absorbancy. He suggested that the difference between the absorbancy values measured at two pH levels is truer representation of anthocyanins content.

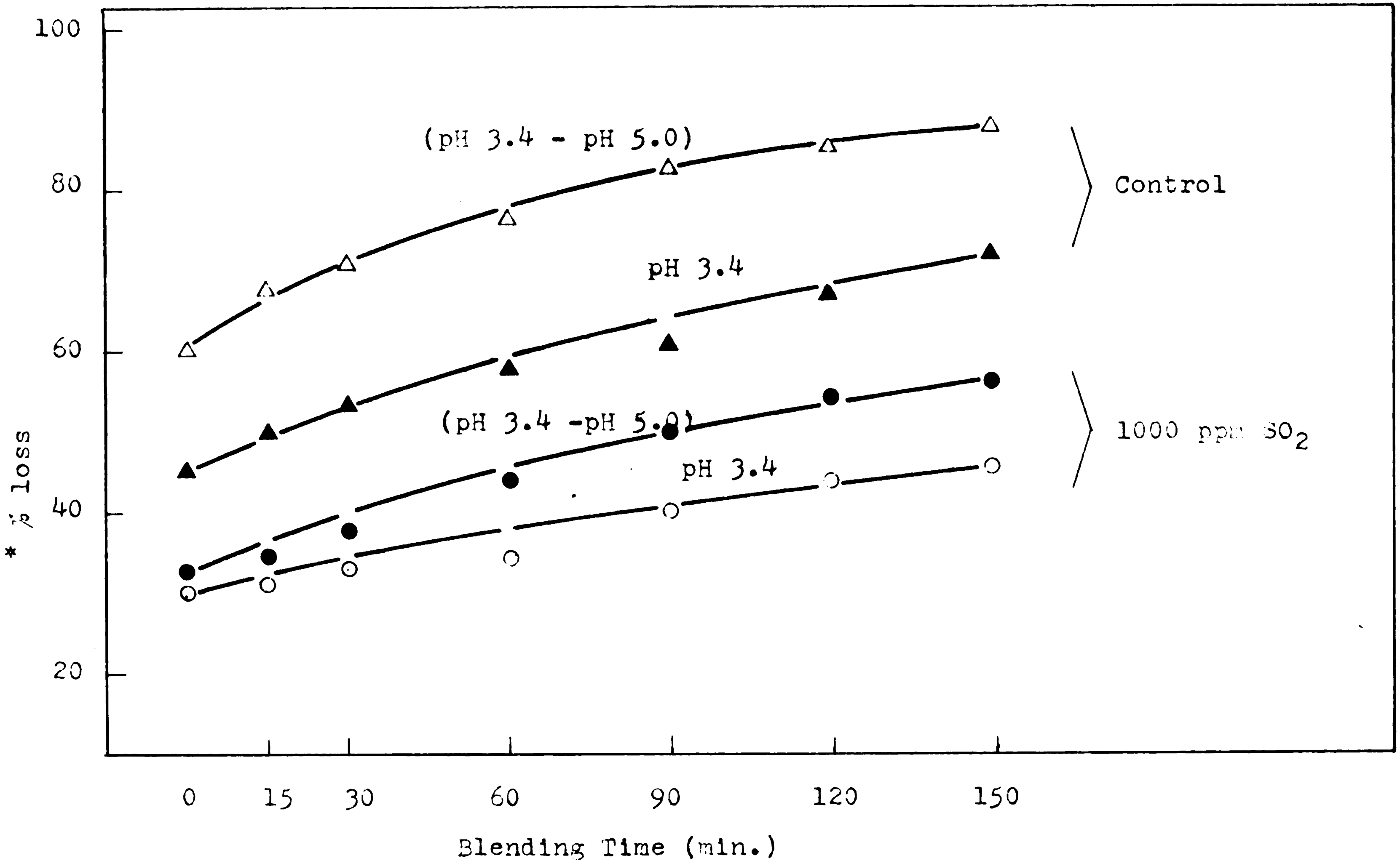
Figure 2 shows the relationships between absorbancy values obtained at pH 3.4 and those obtained as a difference between pH 3.4 and pH 5.0. Although the absorbancy difference value indicated higher % loss of red pigment as compared to absorbancy measured at one pH level, the rates of destruction do not differ appreciably.

These results indicated that absorbancy determination at one pH were suitable for measuring rate of red color destruction. The percent loss of red color and the percent loss during blending calculated on the basis of the two procedures are given in table 5, for the control and 1000 ppm SO_2 treated fruit. The red color losses based on the absorbancy values measured at pH 3.4 were 72 and 46 percent respectively for the control and 1000 ppm SO_2 pretreated fruit after 150 minutes of blending and those based on the pH difference measurement were 87 and 56 percent.

The rate of red color loss in the various treatments after 240 days of frozen storage under accelerated oxidative defrosting conditions is shown in figure 3 (data appendix table 4). The cherries pretreated with 1000 ppm SO_2 showed the least red color loss follow by 500 ppm SO_2, citric acid and control, sirup and ascorbic acid-citric acid.

The sugar sirup packed cherries while retaining the highest red color throughout storage period, under highly oxidative conditions showed very rapid red color destruction.

Figure 2: Comparative discoloration rates of red color, absorbancy at pH 3.4 and as difference between pH 3.4 and pH 5.0.



* % loss, calculated as related to initial value after freezing (1.700)

Table 5: Comparative discoloration rates in red color absorbancy at pH 3.4 and as difference between pH 3.4 and pH 5.0.

	Control						SO_2 1000 ppm					
	(pH 3.4)			Difference (pH 3.4 - pH 5.0)			(pH 3.4)			Difference (pH 3.4-pH 5.0)		
Blending Time (min.)	A_{515} *	% loss **	% loss during blending ***	A_{515}	% loss	% loss during blending	A_{515}	% loss	% loss during blending	A_{515}	% loss	% loss during blending
0	0.931	45.[illegible]	0.00	0.375	59.77	0.00	1.200	29.41	0.[illegible]	0.623	32.62	0.00
15	0.840	50.59	9.27	0.322	65.45	14.13	1.171	31.12	2.42	0.610	34.55	2.86
30	0.801	52.89	14.00	0.274	70.60	26.94	1.133	33.35	5.58	0.590	36.70	6.05
60	0.734	56.83	21.16	0.226	75.75	39.74	1.070	37.06	10.83	0.523	43.89	16.76
90	0.663	61.00	28.79	0.164	82.40	56.27	1.012	40.47	15.67	0.470	49.57	25.15
120	0.561	67.00	39.74	0.139	85.09	62.94	0.960	43.53	20.00	0.427	54.19	32.00
150	0.475	72.06	48.97	0.116	87.55	69.01	0.922	45.77	23.17	0.403	56.76	35.82
180							0.883	48.06	26.42	0.326	65.03	48.08

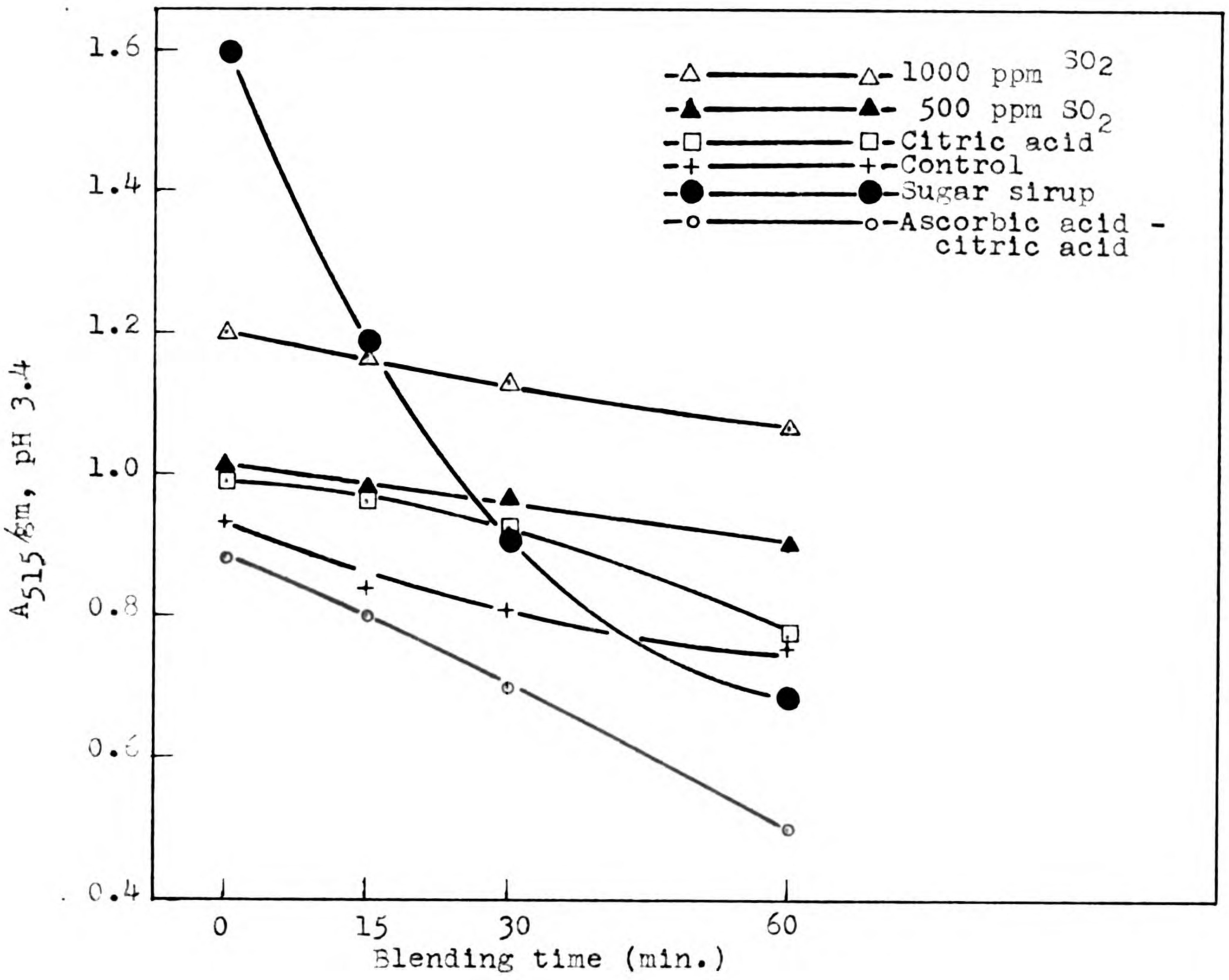
* Absorbancy units at 515 mu per gram of frozen cherries.

** % loss, calculated as related to initial value after freezing (1.700).

*** % loss during blending, calculated as related to initial value at 0-time of blending (0.932).

Values are averages of 4 replicates.

Figure 3: Effect of various treatment on the rate of red color destruction during accelerated oxidative defrosting conditions.



The percent loss of red color for the various treatments calculated on the basis of the two procedures is given in table 6. The difference in percent red color loss between the two methods for the control, SO_2 and citric acid pretreated cherries are relatively constant for each of the blending periods and are about 17, 10, 3 and 11% higher respectively for the losses determined by the difference method. The differences in the losses determined by the methods increased with blending time for the ascorbic acid - citric acid pretreated fruit which might be due to increased formation of brown pigments as pointed out by Hodge (1953). No explanation could be given to the increasing difference between the two red color measuring methods as time of blending of the sirup packed fruit increased. The percent loss of anthocyanins after 240 days of frozen storage was 6, 32, 50, 52, 61 and 62 respectively for the sirup packed, 1000 ppm SO_2, 500 ppm SO_2, citric acid, ascorbic acid - citric and control fruit. After 60 minutes of blending the losses were 86, 43, 58, 67, and 98 percent respectively.

Effect of containers

The cherries stored in the 30 lbs. friction top cans showed less red color loss and browning than those stored in 1 lb. polyethylene bags for all treatments (figure 4), and (data appendix table 4). In addition the rate of red color loss under accelerated conditions of oxidation was lower for

Table 6: Percent color loss based on absorbancy values determined at pH 3.4 and by difference between pH 3.4 and pH 5.0.

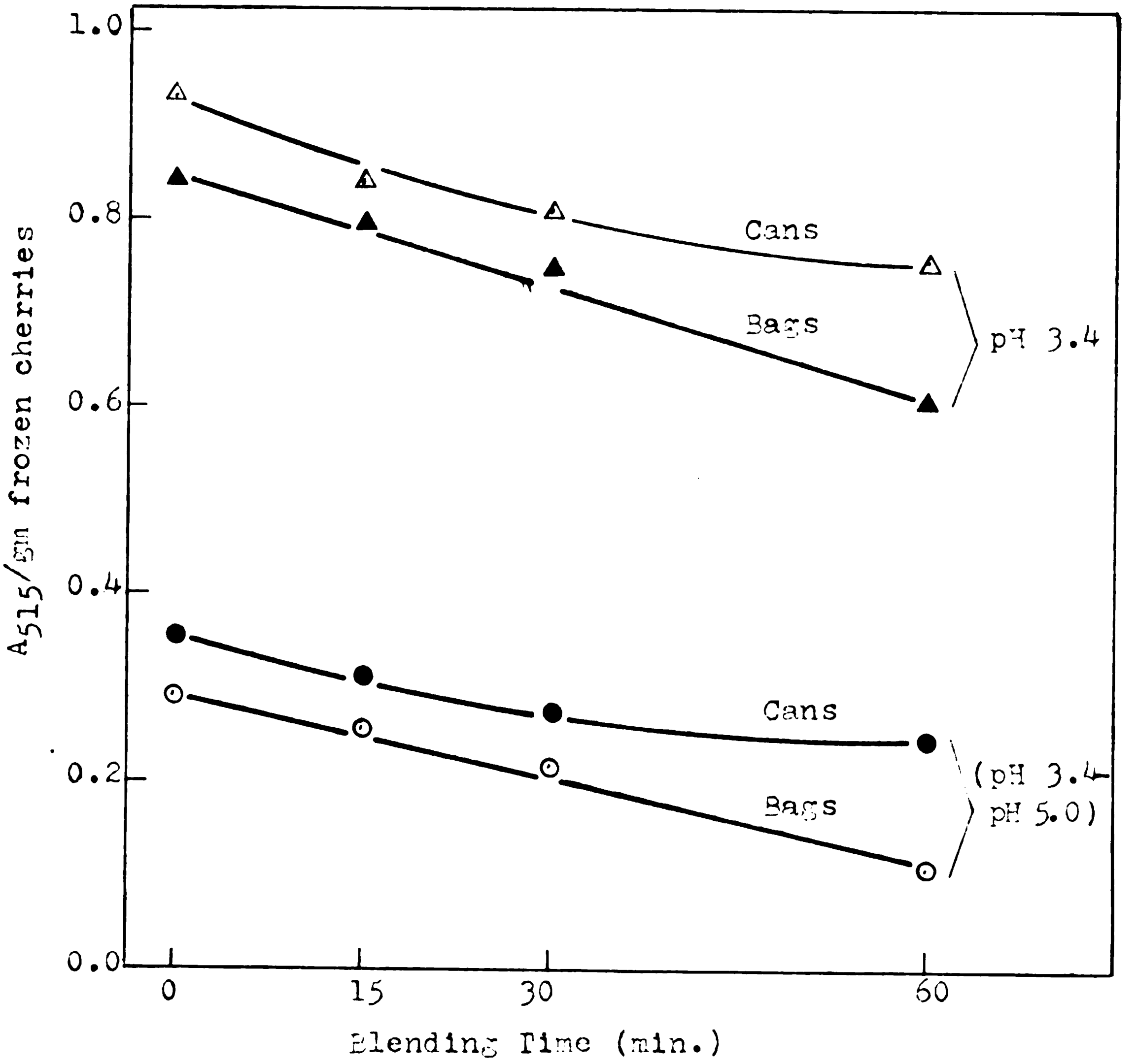
Blending time (min.)	0		15		30		60	
	(pH 3.4)	Difference (pH 3.4- pH 5.0)	(pH 3.4)	Difference (pH 3.4- pH 5.0)	(pH 3.4)	Difference (pH 3.4- pH 5.0)	(pH 3.4)	Difference (pH 3.4- pH 5.0)
Treatment	% loss*	% loss**	% loss*	% loss**	% loss	% loss	% loss	% loss
Control	45.23	61.90	50.59	65.45	52.29	70.60	55.41	73.71
SO_2 500 ppm	40.41	50.00	42.41	52.14	42.94	51.60	47.47	58.26
SO_2 1000 ppm	29.41	32.51	31.12	34.54	33.35	36.69	37.06	43.34
Citric acid 0.1%	41.82	52.14	42.70	53.21	45.53	55.68	54.82	67.59
Ascorbic - citric acid (0.1% - 0.1%)	48.06	61.37	52.56	68.02	58.76	76.39	70.64	97.96
60% sugar sirup	6.23	6.22	30.23	44.85	46.35	69.10	59.88	86.16

* % loss, calculated as related to initial value after freezing (1.700).

** % loss, calculated as related to initial value after freezing (0.932).

The values are averages of 4 replicates.

Figure 4: Effect of container on red color degradation in control cherries, under accelerated oxidation conditions. (Absorbancy at pH 3.4 and as difference between pH 3.4 and pH 5.0)



the fruit stored in the 30 lbs cans. These results are in agreement with those of Guadagni et al. (1957), who reported much less red color loss of red tart cherries in hermetically sealed containers as compared to metal and paperboard containers. Although the friction top cans were not hermetically sealed, the amount of oxygen available would be less per unit of fruit than in the polyethylene freezer bags. It was also found that the bags were not completely vapor proof and therefore would permit a continual supply of air to the product due to breathing action during storage. This is also amply demonstrated by the fact that fruit packed in sirup in hermatically sealed containers showed very little loss of red color.

The increased rate of the red color destruction in the fruit packed in bags could be due to a greater accumulation of quinonees during frozen storage. Scheiner (1960) and Peng and Markakis (1963) have proposed that the main destruction of anthocyanin is by quinones and that the amount of quinone accumulation is dependent upon oxygen availability.

The oxidation of the phenolic compounds in the various treatments showed higher degree of oxidation (lower values) in the cherries packed in 1 lb. polyethylene bags as compared to those packed in 30 lbs. friction top cans. (table 7).

Table 7: Effect of various treatments and different packages on the oxidation of phenolic compounds.

	30 lbs Cans				1 lb. polyethylene bags			
Blending time (min.)	0	15	30	60	0	15	30	60
Treatments	A_{660mu}/gm frozen cherries							
Control	3.660	3.348	3.312	2.976	3.180	2.892	2.760	2.364
SO_2 500 ppm	4.032	3.720	3.672	3.324	3.216	3.132	3.096	2.712
SO_2 1000 ppm	4.260	4.080	3.960	3.624	3.444	3.404	3.384	3.180
Citric acid	3.216	3.036	3.060	2.688	3.240	3.072	2.964	2.484
Ascorbic-citric acid	3.648	3.432	3.024	2.340	2.805	2.544	2.508	2.100
Sirup packed	5.904	4.872	4.020	3.432				

The values are averages of 4 replicates.

Sulfur dioxide Determination

Since SO_2 proved to be effective in decreasing the loss of red color and browning of the fruit, it seemed desireable to determine the residual amount present in the fruit. No objectionable flavor could be detected by sensory evaluation and there was no visual bleaching of the anthocyanins.

No SO_2 could be recovered from any of the SO_2 treated fruit either by direct acid hydrolysis (Monier-Williams distillation method), or by the combination of saponification with sodium hydroxide and acid hydrolysis. Distillation of known concentration of SO_2 solutions gave recoveries of 98%.

These results indicated that either the SO_2 had been oxidized as proposed by Ponting (1960), or it had combined in such a way that it was not released during distillation.

In experiments to study the unrecoverable SO_2 cherries of both control samples and those treated with 1000 ppm SO_2 prior to freezing were stored in SO_2 solutions for 16 hours at 32°F. The results obtained showed that from 1.6 to 2.2 mg of SO_2 (32 to 44 ppm) retained by 50 gm of frozen cherries and not released during distillation (table 8).

Table 8: Milligram SO_2 recovered from frozen cherries stored with known amount of SO_2.

mg SO_2 added	mg SO_2 recovered	Difference
Control		
8.00 (0.00)[1]	6.08 (0.32)	1.92
15.68 (0.64)	13.44 (1.28)	2.24
30.72 (1.28)	29.12 (1.60)	1.60
SO_2 pretreated cherries		
7.68 (0.00)	6.08 (0.32)	1.60
16.32 (0.00)	14.40 (0.32)	1.92

[1] The range of concentrations.

The values are averages of 5 replicates.

Burton and McWeeny (1963) suggested that SO_2 reacts with unsaturated aldehydes forming β-sulfonaldehydes, which will not release SO_2 upon acid hydrolysis. This appears to be a likely mechanism leading to a reduction in the amount of measurable sulfite in stored food stuff.

Effect of added SO_2 on color loss and oxidation under accelerated conditions.

Since the cherries pretreated with SO_2 prior to freezing lost some of their red color and browned under accelerated conditions, it was of interest to determine the amount of SO_2 required to prevent discoloration during blending.

Control as well as fruit pretreated with 1000 ppm SO_2 were used. Sufficient 0.3% $NaHSO_3$ solution was added to obtain 12 and 25 ppm of SO_2 in the mixture. Twenty five ppm SO_2

prevented red color destruction during 60 minutes of blending in the control samples, but did not prevent the oxidation of the phenolic compounds, although the rate was lower than without addition of SO_2. Higher total phenolic compounds values were obtained upon addition of SO_2 (tables 9 and 10).

No explanation can be given for the increased phenolic compound value. The absorbancy values obtained using a pure 25 ppm SO_2 solution (blank), indicate that neither the increase nor the reduction in phenolic compounds value during blending could be accounted for by the reaction between the Folin-Denis reagent and SO_2. One might speculate, the addition compounds of quinones and SO_2, hydroxysulfonates, might react with the Folin-Denis reagent. However this is questionable since similar addition of SO_2 to the cherries pretreated with 1000 ppm SO_2 did not give an increase in phenolic compounds value.

Twelve ppm SO_2 added during blending almost completely prevented red color destruction and phenolic compounds oxidation in the 1000 ppm SO_2 pretreated cherries; 25 ppm SO_2 bleached the cherries. Both 12 and 25 ppm SO_2 prevented the oxidation of the phenolic compounds during blending. These results indicate that it might be more desireable to use slightly higher concentrations of SO_2 in the pretreatment of the cherries prior to freezing.

Table 9: Effect of added SO_2 on color loss under accelerated oxidative defrosting conditions.

Sample	Control		SO_2 1000 ppm		
SO_2 added in (ppm)	0	25	0	12	25
Blending time (min.)	A_{515} mu/gm frozen cherries				
0	1.128[1]	1.123	1.411	1.382	1.166
15	1.070	1.114	1.334	1.382	1.152
30	0.974	1.128	1.248	1.363	1.195
60	0.725	1.114	1.138	1.320	1.186

The values are averages of 4 replicates

Table 10: Effect of added SO_2 on phenolic compound oxidation under accelerated oxidative defrosting conditions.

Sample	Control		SO_2 1000 ppm			H_2O
SO_2 added in (ppm)	0	25	0	12	25	25
Blending time (min.)	A_{660} mu/gm frozen cherries					
0	3.696[1]	4.104	3.816	3.744	3.780	0.079 (0.053)[2]
15	3.480	4.008	3.684	3.756	3.768	0.053 (0.053)
30	3.048	3.864	3.504	3.684	3.816	0.000
60	2.338	3.492	3.252	3.660	3.756	0.000

[2] The range of values

The values are averages of 4 replicates

SUMMARY

The effect of various chemical additives as to their efficiency in preventing red color degradation and phenolic compounds oxidation in red tart cherries frozen by individually quick freezing procedure was studied.

Color loss and phenolic compounds oxidation occurred throughout the 8 months storage period in all the treatments. The cherries pretreated with 1000 ppm SO_2 showed the least color loss, followed by the 500 ppm SO_2 pretreated cherries. The citric acid treatment gave results similar to the control, ascorbic acid - citric acid pretreated cherries had greater red color loss than those of control. Cherries frozen in 60% sugar sirup showed little or no red color loss during frozen storage. The oxidation of phenolic compounds followed the same trend.

Under accelerated oxidative defrosting conditions red color loss and oxidation or browning occurred in cherries in all treatments and the rate of change followed the same order as found for the treatments during frozen storage. Cherries frozen in 60% sugar sirup showed a very rapid rate of red color destruction and oxidation of phenolic compounds with increasing blending time.

The various pretreated cherries packed in 30 lbs. friction top cans retained much better red color than those packed in 1 lb. polyethylene bags. In addition the rate of anthocyanin pigments destruction under accelerated oxidative

defrosting conditions was greater in the cherries packed in 1 lb. polyethylene bags.

Pretreatment of the pitted fruit with SO_2 in the concentration used prove to be very effective in the retardation of red color loss and browning. No SO_2 could be recovered in any of the SO_2 treated fruit by direct acid hydrolysis (Moneir-Williams distillation method), or combination of saponification with sodium hydroxide and hydrolysis with acid.

Experiments with addition of SO_2 just before defrosting to further improve red color retention under accelerated oxidation conditions showed that a concentration of 25 ppm SO_2 prevented red color destruction during 60 minutes of blending in the control samples, but did not prevent phenolic compounds oxidation, although the rate was half that without SO_2. Twelve ppm SO_2 practically prevented red color destruction and phenolic compounds oxidation in the cherries pretreated with 1000 ppm SO_2 prior to freezing. The addition of 25 ppm SO_2 bleached the fruit.

In conclusion SO_2 found to be effective in decreasing red color degradation and browning during frozen storage and subsequent defrosting. The concentration used did not impart an undesireable flavor or cause color bleaching. The results indicated that a slightly higher SO_2 concentration may be used for pretreating the fruit prior to freezing for better color retention.

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APPENDIX

Table 1: Stability of red color absorbancy at pH 3.4 and as difference between pH 3.4 and pH 5.0 after addition of 70% alcohol.

Time after alcohol added \ Blending time	0 min.		30 min.	
	pH 3.4 A_{515}/gm	Difference (pH 3.4 - pH 5.0) A_{515}/gm	pH 3.4 A_{515}/gm	Difference (pH 3.4 - pH 5.0) A_{515}/gm
120 min.	1.286	0.691	1.032	0.600
	1.238	0.638	1.022	0.600
	1.248	0.634	1.032	0.557
	1.267	0.638	1.003	0.552
160 min.	1.258	0.672	1.013	0.571
	1.267	0.667	1.003	0.566
	1.286	0.682	0.998	0.552
	1.258	0.658	0.979	0.595
180 min.	1.258	0.652	0.979	0.566
	1.277	0.648	1.003	0.571
	1.286	0.672	1.013	0.557
	1.286	0.686	0.989	0.586
200 min.	1.248	0.648	0.979	0.576
	1.286	0.682	0.979	0.571
	1.277	0.682	0.989	0.566
	1.277	0.648	0.998	0.571

Table 2: Stability of red color values after addition of sodium-citrate buffer pH 3.4 and pH 5.0.

Time After the Buffer Added	Blending time (min) 0		15		30		60	
	(pH 3.4) A_{515}/gm*	(pH 5.0) A_{515}/gm	(pH 3.4) A_{515}/gm	(pH 5.0) A_{515}/gm	(pH 3.4) A_{515}/gm	(pH 5.0) A_{515}/gm	(pH 3.4) A_{515}/gm	(pH 5.0) A_{515}/gm
15 min.	1.200 1.171	0.614 0.605	1.109 1.090	0.576 0.586	0.970 0.965	0.557 0.547	0.658 0.662	0.466 0.446
30 min.	1.190 1.171	0.619 0.605	1.099 1.085	0.576 0.586	0.965 0.955	0.557 0.547	0.658 0.662	0.470 0.446
45 min.	1.219 1.181	0.619 0.605	1.109 1.099	0.586 0.586	0.979 0.965	0.557 0.538	0.672 0.677	0.470 0.446
60 min.	1.190 1.162	0.619 0.605	1.090 1.075	0.576 0.576	0.965 0.946	0.547 0.528	0.648 0.658	0.466 0.442
75 min.	1.190 1.162	0.614 0.605	1.090 1.066	0.576 0.576	0.946 0.941	0.547 0.528	0.648 0.634	0.466 0.442
90 min.	1.162 1.142	0.619 0.605	1.090 1.056	0.576 0.576	0.941 0.931	0.547 0.528	0.643 0.629	0.451 0.442

*Absorbancy at 515 mu per gram frozen cherries.

Table 3: Red color loss during storage time for the various treatments.

Time (days)	Control		SO_2 500 ppm		SO_2 1000 ppm		Ascorbic-citric acid		Citric acid		60% sugar sirup	
	A_{515}	% ** loss	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss
0	1.700											
30	1.510	11.18	1.562	8.12	1.664	2.12	1.303	23.50	1.453	14.53		
34	1.126	33.76	1.203	29.23	1.433	15.70	0.973	42.76	0.989	41.82		
180	0.974	42.70	1.171	31.12	1.296	23.76						
240	0.931	45.23	1.013	40.41	1.200	29.41	0.883	48.06	0.973	42.76	1.594	6.23

* Absorbancy at 515 mu calculated per gram frozen cherries

** % loss calculated as related to initial color value of pitted cherries after freezing.

The values are averages of 4 replicates.

Table 4: Effect of various treatment and containers on the rate of red color. (Absorbancy determined at pH 3.4 and by difference between pH 3.4 and pH 5.0.)

CONTROL

	CANS				POLYTHYLENE BAGS			
Time	(pH 3.4)		Difference (pH 3.4 - pH 5.0)		(pH 3.4)		Difference (pH 3.4 - pH 5.0)	
(min.)	A_{515}*	% loss**	A_{515}	% loss***	A_{515}	% loss	A_{515}	% loss
0	0.931	45.23	0.355	61.90	0.840	50.59	0.293	68.56
15	0.840	50.59	0.322	65.45	0.797	53.12	0.235	74.78
30	0.811	52.29	0.274	70.60	0.744	56.23	0.221	76.28
60	0.758	55.41	0.245	73.71	0.610	64.11	0.106	88.62
% loss during blending	18.58		31.00		27.38		63.82	

* Absorbancy units at 515 mu per gram of frozen cherries

** % loss, calculated as related to initial value after freezing (1.700)

*** % loss, calculated as related to initial value after freezing (0.932)

Table 4: (continued)

SO$_2$ 500 ppm

Time	CANS				POLYETHYLENE BAGS			
	(pH 3.4)		Difference (pH 3.4 - pH 5.0)		(pH 3.4)		Difference (pH 3.4 - pH 5.0)	
(min.)	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss
0	1.013	40.41	0.466	50.00	0.859	49.47	0.307	67.07
15	0.979	42.41	0.446	52.14	0.850	50.00	0.254	72.74
30	0.970	42.94	0.451	51.60	0.811	52.29	0.254	72.74
60	0.893	47.47	0.389	58.26	0.701	58.76	0.125	86.58
% loss during blending	11.85		16.52		18.39		59.28	

Table 4: (continued)

SO$_2$ 1000 ppm

Time	CANS				POLYETHYLENE BAGS			
	(pH 3.4)		Difference (pH 3.4 - pH 5.0)		(pH 3.4)		Difference (pH 3.4 - pH 5.0)	
(min.)	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss
0	1.200	29.41	0.629	32.51	0.974	42.70	0.384	58.79
15	1.171	31.12	0.610	34.54	0.946	44.35	0.346	62.87
30	1.133	33.35	0.590	36.69	0.907	45.65	0.331	64.48
60	1.070	37.06	0.528	43.34	0.749	55.94	0.182	80.47
% loss during blending	10.83		16.05		23.00		52.60	

Table 4: (continued)

ASCORBIC ACID - CITRIC ACID

	CANS				POLYETHYLENE BAGS			
Time	(pH 3.4)		Difference (pH 3.4 - pH 5.0)		(pH 3.4)		Difference (pH 3.4 - pH 5.0)	
(min.)	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss
0	0.883	48.06	0.360	61.37	0.739	56.53	0.197	78.86
15	0.806	52.56	0.298	68.02	0.691	59.35	0.115	87.66
30	0.701	58.76	0.220	76.39	0.624	63.29	0.096	89.69
60	0.499	70.64	0.019	97.96	0.389	77.11	0.014	98.49
% loss during blending	43.48		94.72		47.36		92.89	

Table 4: (continued)

CITRIC ACID

	CANS				POLYETHYLENE BAGS			
Time	(pH 3.4)		Difference (pH 3.4 - pH 5.0)		(pH 3.4)		Difference (pH 3.4 - pH 5.0)	
(min.)	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss	A_{515}	% loss
0	0.989	41.82	0.446	52.14	0.878	48.35	0.360	61.37
15	0.974	42.70	0.437	53.21	0.840	50.59	0.346	62.87
30	0.926	45.53	0.413	55.68	0.806	52.59	0.317	65.98
60	0.768	54.82	0.302	67.59	0.706	58.47	0.274	70.60
% loss during blending	22.34		32.28		19.58		23.89	

Table 4: (Continued)

<u>60% sugar sirup</u>

303 Cans

Time	(pH 3.4)		Difference (pH 3.4 - pH 5.0)	
(min.)	A_{515}	% loss	A_{515}	% loss
0	1.594	6.23	0.874	6.22
15	1.186	30.23	0.514	44.85
30	0.912	46.35	0.288	69.10
60	0.682	59.88	0.129	86.16
% loss during blending	57.22		85.25	